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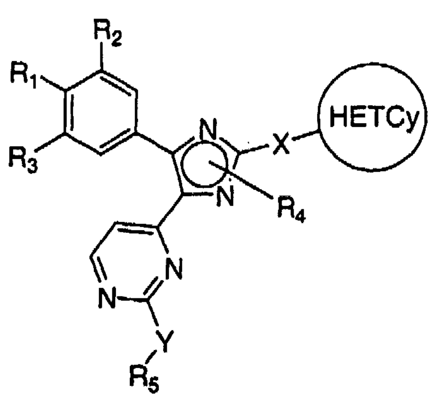
(54) **Title:** USE OF (DI-SUBSTITUTED-PHENYL)-PYRIMIDINYL-IMIDAZOLE DERIVATIVES AS JNK-INHIBITORS



(I)

(57) Abstract: A method of promoting neuronal survival and helping prevent neuronal death administers (di-substituted-phenyl) pyrimidinyl imidazole derivative compounds represented by formula (I) effective to inhibit the activity of c-jun-*N*-terminal kinase.

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TITLE OF THE INVENTION

USE OF (DI-SUBSTITUTED-PHENYL)-PYRIMIDINYL-IMIDAZOLE DERIVATIVES AS JNK-INHIBITORS

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention is directed to a method of use of (di-substituted-phenyl) pyrimidinyl imidazole derivative compounds to inhibit c-jun-*N*-terminal kinase. In particular, this invention is directed to a method of use of (di-substituted-phenyl) pyrimidinyl imidazole derivative compounds to promote neuronal survival and help prevent neuronal death by inhibiting c-jun-*N*-terminal kinase.

RELATED BACKGROUND

Extracellular stimuli can cause a wide range of responses from the cell receiving such stimuli. One common response is the expression by the cell of specific proteins functionally responsive to the stimulus. There are, however, many intermediate steps between a stimulus and the resulting responsive expression of protein. The stimulus/response processes typically follow pathways (cascades) that are mediated at each step by enzymes, the presence of which facilitates the step. Conversely, the absence of a mediating enzyme can suppress the step, thereby suppressing the response.

Humans are composed of cells and some cellular responses can cause problems for people. For example, neuronal death can result from apoptosis caused by a cellular response to stress. Thus, it would be desirable to provide a method of preventing neuronal death and promote neuronal survival by inhibiting a cellular response detrimental to neurons.

As described in, for example, Y.T. Ip and R.J. Davis, *Curr. Opin. Cell Biol.*, 10:205-219 (1998) and A. Minden and M. Karin, *Biochimica et Biophysica Acta*, 1333:F85-F104 (1997), certain stimuli that include stress, UV radiation, and cytokines can initiate a cascade which leads to the phosphorylation of the transcriptional activation domains of the transcription factor c-Jun. The phosphorylation of c-Jun is mediated by c-Jun *N*-terminal kinase ("JNK") which is a

mitogen-activated protein kinase ("MAP kinase" or "MAPK"). The transcription factor c-Jun has been implicated in cell proliferation, cell differentiation, and neoplastic transformation. It has been speculated that JNK might play a role in cellular apoptosis. Thus, it would be desirable to provide a method of preventing cellular apoptosis by inhibiting the appropriate MAP kinase that mediates the apoptosis cellular response.

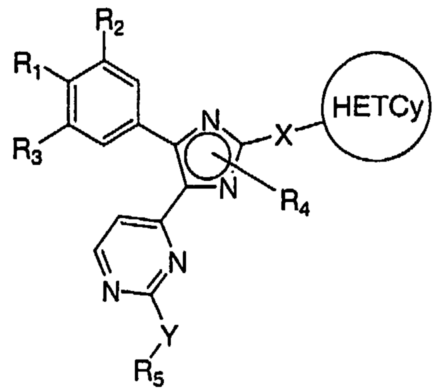
U.S. Patent Nos. 5,736,381 and 5,804,427 describe cytokine, stress, and oncoprotein activated human kinase kinases. U.S. Patent Nos. 5,717,100, 5,859,041, 5,783,664, 5,955,366, UK Patent Publication GB 2 336 362, and International Patent Publication WO 99/47512, WO 97/33883, and WO 98/24782 describe various methods of treatment by the inhibition of cytokines and compounds that inhibit cytokines. The compounds utilized by the method of the present invention are described in U.S. Patent No. 5,859,041. However, cytokine stimulus can produce responses other than neuronal distress, such as inflammation. Further, as described above, neuronal distress can result from celluar responses to stimuli other than cytokines. Thus, it would be desirable to provide a method of preventing neuronal distress by inhibiting the appropriate MAP kinase further downstream from the stimulus and more proximate to the response detremental to neurons. Such a method can provide better specificity with fewer unwanted side effects.

BRIEF DESCRIPTION OF THE FIGURES

Fig.1 is a graphical plot of the %inhibition vs. concentration of an Example of the invention.

SUMMARY OF THE INVENTION

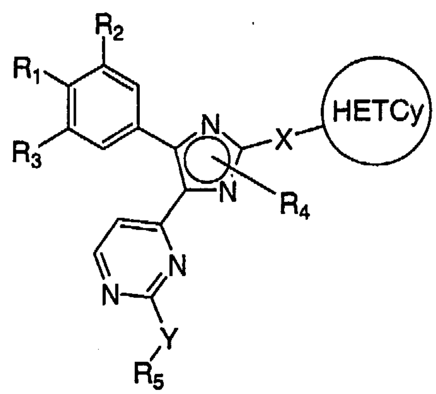
The present invention promotes neuronal survival by an administration of an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase:



(I)

DETAILED DESCRIPTION OF THE INVENTION

A method of this invention promotes neuronal survival by an administration of an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase:



(I)

wherein

R_1 is –F, –Cl, –Br, -OH, –SH, –NH_2, or –CH_3;

R_2 is –F, –Cl, –Br, -OH, –SH, –NH_2, or –CH_3;

R_3 is –H, –F, –Cl, –Br, -OH, –SH, –NH_2, –CH_3, –OCH_3, or –CH_2CH_3;

R_4 is –C_{1-4}alkyl optionally substituted with a –C_{3-7}cycloalkyl;

R_5 is $-C_{1-4}$alkyl or $-C_{3-7}$cycloalkyl, wherein the $-C_{1-4}$alkyl is optionally substituted with a phenyl;

X is a bond or an alkyl bridge having 1-3 carbons;

Y is –NH– or $-NH_2^+-$; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1-4}$alkyl or –C(O)–O–CH_2phenyl.

In one aspect, a method of this invention administers an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase, wherein

R_1 is –Cl;

R_2 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_3 is –H, –F, –Cl, –Br, -OH, –SH, $-NH_2$, $-CH_3$, $-OCH_3$, or $-CH_2CH_3$;

R_4 is $-C_{1-4}$alkyl optionally substituted with a $-C_{3-7}$cycloalkyl;

R_5 is $-C_{1-4}$alkyl or $-C_{3-7}$cycloalkyl, wherein the $-C_{1-4}$alkyl is optionally substituted with a phenyl;

X is a bond or an alkyl bridge having 1-3 carbons;

Y is –NH– or $-NH_2^+-$; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1-4}$alkyl or –C(O)–O–CH_2phenyl.

In an embodiment of this aspect, a method of this invention administers an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase, wherein

R_1 is –Cl;

R_2 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_3 is –H;

R_4 is $-C_{1-4}$alkyl optionally substituted with a $-C_{3-7}$cycloalkyl;

R_5 is $-C_{1-4}$alkyl or $-C_{3-7}$cycloalkyl, wherein the $-C_{1-4}$alkyl is optionally substituted with a phenyl;

X is a bond or an alkyl bridge having 1-3 carbons;

Y is –NH– or $-NH_2^+-$; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1-4}$alkyl or –C(O)–O–CH_2phenyl.

In another embodiment of this aspect, a method of this invention administers an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase, wherein

R_1 is –Cl;

R_2 is –Cl;

R_3 is –H;

R_4 is $-C_{1-4}$alkyl optionally substituted with a $-C_{3-7}$cycloalkyl;

R_5 is $-C_{1-4}$alkyl or $-C_{3-7}$cycloalkyl, wherein the $-C_{1-4}$alkyl is optionally substituted with a phenyl;

X is a bond or an alkyl bridge having 1-3 carbons;

Y is –NH– or $-NH_2^+-$; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1-4}$alkyl or –C(O)–O–CH_2phenyl.

In a second aspect, a method of this invention administers an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase, wherein

R_1 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_2 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_3 is –H, –F, –Cl, –Br, -OH, –SH, $-NH_2$, $-CH_3$, $-OCH_3$, or $-CH_2CH_3$;

R_4 is $-C_{1-4}$alkyl optionally substituted with a $-C_{3-7}$cycloalkyl;

R_5 is $-C_{1-4}$alkyl or $-C_{3-7}$cycloalkyl, wherein the $-C_{1-4}$alkyl is optionally substituted with a phenyl;

X is a bond;

Y is –NH– or $-NH_2^+-$; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1-4}$alkyl or $-C(O)-O-CH_2$phenyl.

In an embodiment of this aspect, a method of this invention administers an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase, wherein

R_1 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_2 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_3 is –H, –F, –Cl, –Br, -OH, –SH, $-NH_2$, $-CH_3$, $-OCH_3$, or $-CH_2CH_3$;

R_4 is $-C_{1-4}$alkyl optionally substituted with a $-C_{3-7}$cycloalkyl;

R_5 is $-C_{1-4}$alkyl or $-C_{3-7}$cycloalkyl, wherein the $-C_{1-4}$alkyl is optionally substituted with a phenyl;

X is a bond;

Y is –NH–; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1-4}$alkyl or $-C(O)-O-CH_2$phenyl.

In another embodiment of this aspect, a method of this invention administers an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase, wherein

R_1 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_2 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_3 is –H, –F, –Cl, –Br, -OH, –SH, $-NH_2$, $-CH_3$, $-OCH_3$, or $-CH_2CH_3$;

R_4 is $-C_{1-4}$alkyl optionally substituted with a $-C_{3-7}$cycloalkyl;

R_5 is $-C_{1-4}$alkyl, optionally substituted with a phenyl;

X is a bond;

Y is –NH–; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1-4}$alkyl or $-C(O)-O-CH_2$phenyl.

In still another embodiment of this aspect, a method of this invention administers an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase, wherein

R_1 is $-F$, $-Cl$, $-Br$, -OH, $-SH$, $-NH_2$, or $-CH_3$;

R_2 is $-F$, $-Cl$, $-Br$, -OH, $-SH$, $-NH_2$, or $-CH_3$;

R_3 is $-H$, $-F$, $-Cl$, $-Br$, -OH, $-SH$, $-NH_2$, $-CH_3$, $-OCH_3$, or $-CH_2CH_3$;

R_4 is $-C_{1-4}$alkyl optionally substituted with a $-C_{3-7}$cycloalkyl;

R_5 is $-C_3$cycloalkyl;

X is a bond;

Y is $-NH-$; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1-4}$alkyl or $-C(O)-O-CH_2$phenyl.

In yet another embodiment of this aspect, a method of this invention administers an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase, wherein

R_1 is $-F$, $-Cl$, $-Br$, -OH, $-SH$, $-NH_2$, or $-CH_3$;

R_2 is $-F$, $-Cl$, $-Br$, -OH, $-SH$, $-NH_2$, or $-CH_3$;

R_3 is $-H$, $-F$, $-Cl$, $-Br$, -OH, $-SH$, $-NH_2$, $-CH_3$, $-OCH_3$, or $-CH_2CH_3$;

R_4 is $-C_{1-4}$alkyl optionally substituted with a $-C_{3-7}$cycloalkyl;

R_5 is $-C_6$cycloalkyl;

X is a bond;

Y is $-NH-$; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1-4}$alkyl or $-C(O)-O-CH_2$phenyl.

In another embodiment of this aspect, a method of this invention administers an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase, wherein

R_1 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_2 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_3 is –H, –F, –Cl, –Br, -OH, –SH, $-NH_2$, $-CH_3$, $-OCH_3$, or $-CH_2CH_3$;

R_4 is $-C_{1\text{-}4}$alkyl optionally substituted with a $-C_{3\text{-}7}$cycloalkyl;

R_5 is $-C_3$cycloalkyl;

X is a bond;

Y is $-NH_2^+-$; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1\text{-}4}$alkyl or $-C(O)-O-CH_2$phenyl.

In another aspect, a method of this invention administers an amount of an amine bis trifluoroacetic acid salt of a compound represented by Formula (I).

The method of this invention utilizes a subset of compounds of particular interest described by Formula (I) wherein HETCy represents a 5-6 membered non-aromatic heterocycle with 1-2 nitrogen atoms contained therein. In this subset, HETCy is advantageously a pyrrolidinyl or piperidinyl group, and particularly advantageously a 4-piperidinyl group. Within this subset of compounds, all other variables are as described previously.

As used herein, "alkyl" as well as other groups having the prefix "alk" such as, for example, alkoxy, alkanoyl, alkenyl, alkynyl and the like, means carbon chains which may be linear or branched or combinations thereof. Examples of alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, <u>sec</u>- and <u>tert</u>-butyl, pentyl, hexyl, heptyl and the like. "Alkenyl", "alkynyl" and other like terms include carbon chains containing at least one unsaturated C-C bond.

The term "cycloalkyl" means carbocycles containing no heteroatoms, and includes mono-, bi- and tricyclic saturated carbocycles. Examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like.

The term "C_{0-6}alkyl" includes alkyls containing 6, 5, 4, 3, 2, 1, or no carbon atoms. An alkyl with no carbon atoms is a hydrogen atom substituent.

The term "hetero" unless specifically stated otherwise includes one or more O, S, or N atoms. For example, heterocycloalkyl and heteroaryl include ring systems that contain one or more O, S, or N atoms in the ring, including mixtures of such atoms. The hetero atoms replace ring carbon atoms. Thus, for example, a heterocycloC_5alkyl is a five member ring containing from 5 to no carbon atoms.

The term "optionally substituted" is intended to include both substituted and unsubstituted. Thus, for example, optionally substituted aryl could represent a pentafluorophenyl or a phenyl ring. Further, optionally substituted multiple moieties such as, for example, alkylaryl are intended to mean that the aryl and the aryl groups are optionally substituted. If only one of the multiple moieties is optionally substituted then it will be specifically recited such as "an alkylaryl, the aryl optionally substituted with halogen or hydroxyl."

Compounds described herein contain one or more double bonds and may thus give rise to cis/trans isomers as well as other conformational isomers. The method of the present invention includes the utilization of all such possible isomers as well as mixtures of such isomers.

Compounds described herein can contain one or more asymmetric centers and may thus give rise to diastereomers and optical isomers. The method of the present invention includes the utilization of all such possible diastereomers as well as their racemic mixtures, their substantially pure resolved enantiomers, all possible geometric isomers, and pharmaceutically acceptable salts thereof. The above Formula I is shown without a definitive stereochemistry at certain positions. The method of the present invention includes the utilization of all stereoisomers of Formula I and pharmaceutically acceptable salts thereof. Further, mixtures of stereoisomers as well as isolated specific stereoisomers are also included. During the course of the synthetic procedures used to prepare such compounds, or in using racemization or epimerization procedures known to those skilled in the art, the products of such procedures can be a mixture of stereoisomers.

The compounds utilized by the method of the present invention are described in U.S. Patent No. 5,859,041 and methods of preparation are described therein of the compounds utilized by the method of the present invention.

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids. When the compound of the present invention is acidic, its corresponding salt can be conveniently prepared from pharmaceutically acceptable non-toxic bases, including inorganic bases and organic bases. Salts derived from such inorganic bases include aluminum, ammonium, calcium, copper (ic and ous), ferric, ferrous, lithium, magnesium, manganese (ic and ous), potassium, sodium, zinc and the like salts. Particularly preferred are the ammonium, calcium, magnesium, potassium and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, as well as cyclic amines and substituted amines such as naturally occurring and synthesized substituted amines. Other pharmaceutically acceptable organic non-toxic bases from which salts can be formed include ion exchange resins such as, for example, arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine and the like.

When the compound of the present invention is basic, its corresponding salt can be conveniently prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include, for example, acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid and the like. Particularly preferred are citric, hydrobromic, hydrochloric, maleic, phosphoric, sulfuric, and tartaric acids.

The pharmaceutical compositions of the present invention comprise a compound represented by Formula I (or pharmaceutically acceptable salts thereof) as an active ingredient, a pharmaceutically acceptable carrier and optionally other therapeutic ingredients or adjuvants. The compositions include compositions suitable for oral, rectal, topical, and parenteral (including subcutaneous, intramuscular, and

intravenous) administration, although the most suitable route in any given case will depend on the particular host, and nature and severity of the conditions for which the active ingredient is being administered. The pharmaceutical compositions may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

Creams, ointments, jellies, solutions, or suspensions containing the compound of Formula I can be employed for topical use. Mouth washes and gargles are included within the scope of topical use for the purposes of this invention.

Dosage levels from about 0.01mg/kg to about 140mg/kg of body weight per day are useful in the treatment of conditions such as stroke, Parkinsons disease, Alzheimer's disease, amyotrophiclateral sclerosis, multiple sclerosis, spinal cord injury, head trauma, and seizure which are responsive to JNK inhibition, or alternatively about 0.5mg to about 7g per patient per day. For example, stroke may be effectively treated by the administration of from about 0.01mg to 50mg of the compound per kilogram of body weight per day, or alternatively about 0.5mg to about 3.5g per patient per day. Further, it is understood that the JNK inhibiting compounds of this invention can be administered at prophylactically effective dosage levels to prevent the onset of symptoms associated with the above-recited conditions.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration to humans may conveniently contain from about 0.5mg to about 5g of active agent, compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 percent of the total composition. Unit dosage forms will generally contain between from about 1mg to about 500mg of the active ingredient, typically 25mg, 50mg, 100mg, 200mg, 300mg, 400mg, 500mg, 600mg, 800mg or 1000mg.

It is understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

In practice, the compounds represented by Formula I, or pharmaceutically acceptable salts thereof, utilized by the method of this invention can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The

carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). Thus, the pharmaceutical compositions utilized by the method of the present invention can be presented as discrete units suitable for oral administration such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient. Further, the compositions can be presented as a powder, as granules, as a solution, as a suspension in an aqueous liquid, as a non-aqueous liquid, as an oil-in-water emulsion or as a water-in-oil liquid emulsion. In addition to the common dosage forms set out above, the compound represented by Formula I, or pharmaceutically acceptable salts thereof, may also be administered by controlled release means and/or delivery devices. The compositions may be prepared by any of the methods of pharmacy. In general, such methods include a step of bringing into association the active ingredient with the carrier that constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both. The product can then be conveniently shaped into the desired presentation.

Thus, the pharmaceutical compositions utilized by the method of this invention may include a pharmaceutically acceptable carrier and a compound or a pharmaceutically acceptable salt of Formula I. The compounds of Formula I, or pharmaceutically acceptable salts thereof, can also be included in pharmaceutical compositions in combination with one or more other therapeutically active compounds.

The pharmaceutical carrier employed can be, for example, a solid, liquid, or gas. Examples of solid carriers include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Examples of liquid carriers are sugar syrup, peanut oil, olive oil, and water. Examples of gaseous carriers include carbon dioxide and nitrogen.

In preparing the compositions for oral dosage form, any convenient pharmaceutical media may be employed. For example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like may be used to form oral liquid preparations such as suspensions, elixirs and solutions; while carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like may be used to form oral solid preparations such as powders, capsules and tablets. Because of their ease of administration, tablets and capsules are the preferred oral dosage units whereby solid pharmaceutical carriers

are employed. Optionally, tablets may be coated by standard aqueous or nonaqueous techniques

A tablet containing the composition utilized by the method of this invention may be prepared by compression or molding, optionally with one or more accessory ingredients or adjuvants. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. Each tablet preferably contains from about 0.1mg to about 500mg of the active ingredient and each cachet or capsule preferably containing from about 0.1mg to about 500mg of the active ingredient.

Pharmaceutical compositions utilized by the method of the present invention suitable for parenteral administration may be prepared as solutions or suspensions of the active compounds in water. A suitable surfactant can be included such as, for example, hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Further, a preservative can be included to prevent the detrimental growth of microorganisms.

Pharmaceutical compositions utilized by the method of the present invention suitable for injectable use include sterile aqueous solutions or dispersions. Furthermore, the compositions can be in the form of sterile powders for the extemporaneous preparation of such sterile injectable solutions or dispersions. In all cases, the final injectable form must be sterile and must be effectively fluid for easy syringability. The pharmaceutical compositions must be stable under the conditions of manufacture and storage; thus, preferably should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol and liquid polyethylene glycol), vegetable oils, and suitable mixtures thereof.

Pharmaceutical compositions utilized by the method of the present invention can be in a form suitable for topical use such as, for example, an aerosol, cream, ointment, lotion, dusting powder, or the like. Further, the compositions can be in a form suitable for use in transdermal devices. These formulations may be prepared, utilizing a compound represented by Formula I of this invention, or pharmaceutically acceptable salts thereof, via conventional processing methods. As

an example, a cream or ointment is prepared by mixing hydrophilic material and water, together with about 5 wt% to about 10 wt% of the compound, to produce a cream or ointment having a desired consistency.

Pharmaceutical compositions utilized by the method of this invention can be in a form suitable for rectal administration wherein the carrier is a solid. It is preferable that the mixture forms unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories may be conveniently formed by first admixing the composition with the softened or melted carrier(s) followed by chilling and shaping in moulds.

In addition to the aforementioned carrier ingredients, the pharmaceutical formulations described above may include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface-active agents, thickeners, lubricants, preservatives (including anti-oxidants) and the like. Furthermore, other adjuvants can be included to render the formulation isotonic with the blood of the intended recipient. Compositions containing a compound described by Formula I, or pharmaceutically acceptable salts thereof, may also be prepared in powder or liquid concentrate form.

The compounds and pharmaceutical compositions utilized by the method of this invention have been found to exhibit biological activity as JNK inhibitors. Accordingly, another aspect of the invention is the treatment in mammals of, for example, stroke, Parkinsons disease, Alzheimer's disease, amyotrophiclateral sclerosis, multiple sclerosis, spinal cord injury, head trauma, and seizure – maladies that are amenable to amelioration through inhibition of the activity of JNK – by the method of this invention. The term "mammals" includes humans, as well as other animals such as, for example, rats, mice, monkeys, dogs, cats, horses, pigs, and cattle. Accordingly, it is understood that the treatment of mammals other than humans is the treatment of clinical correlating afflictions to those above recited examples that are human afflictions.

ASSAYS DEMONSTRATING BIOLOGICAL ACTIVITY

Biochemical Determination of Inhibition of JNK3α1

Truncated JNK3α1(amino acids 39-422) was expressed in *E. coli*, purified, and activated *in vitro* by a combination of MKK4 and MKK7 in 129μl of a

buffer containing: 25mM HEPES (Sigma, St. Louis, MO) pH 7.4, 10mM $MgCl_2$ (Sigma), 2mM DTT (Sigma), 20mM β-glycerophosphate (Sigma), 0.1mM Na_3VO_4, 200μM ATP (Sigma), 225nM JNK3α1, and 100nM MKK4 + 100nM GST-MKK7(Upstate Biotechnology, Lake Placid, NY). Activation reactions were incubated at 30°C for 2h.

Enzyme inhibition studies were performed at 30°C for 30min with 0.5μM GST-ATF2, 1μM ATP, 1nM activated JNK3α1 and compound ranging from 30pM to 100μM. Reactions were carried out in 100μL volumes containing the final concentrations of the following: 25mM HEPES (Sigma) pH 7.4; 10mM $MgCl_2$ (Sigma); 2mM DTT (Sigma); 20mM β-glycerophosphate (Sigma); 0.1mM Na_3VO_4 (Sigma); 2μCi [γ-^{33}P]ATP (2000Ci/mmol; 1Ci = 37GBq) (Amersham Pharmacia Biotech, Piscataway, NJ). Reactions were stopped with 100μL 100mM EDTA/ 15mM sodium pyrophosphate. Immobilon™-P 96-well plates (Millipore MAIPNOB™ 10, available from the Millipore Corp., Bedford, MA) were pretreated with 100μL methanol, followed by 100μL 15mM sodium pyrophosphate. Fifty μL of the stopped reaction was spotted in triplicate on the Immobilon™-P 96-well plate. The samples were vacuum-filtered and washed three times each with 100μL 75mM H_3PO_4 to remove unincorporated [γ-^{33}P]ATP. After the third H_3PO_4 wash and a final filtration step to remove H_3PO_4, 50μL of Microscint™-20 (Packard BioScience Ltd., Pangbourne, Berkshire, U.K.) was added to each well and samples were analyzed on a Packard Topcount™ liquid scintillation counter. IC_{50} values were determined by fitting the data to the equation for a four parameter logistic.

Table 1

Compound	IC_{50}(nM)
Example 1	8
Example 2	7
Example 3	48
Example 4	1
Example 5a	40
Example 6a	45
Example 7	4

Effects of Jnk inhibition on dopaminergic neuronal survival, *in vitro* following treatment with the neurotoxin MPP$^+$.

Methods

1. Preparation of mesencephalic dopaminergic neurons.

This protocol produces a yield of dopaminergic neurons of around 0.5 - 1%; this is equivalent to roughly 1000 - 1500 dopaminergic cells in the well. 14 day gestation Sprague-Dawley rats were killed by stunning and exsanguination. Embryos were removed and decapitated, and the ventral mesencephalon dissected from the brain. The tissue was dissociated by trypsin (0.25% in Hank's BSS) digestion for 20 minutes. The trypsin was neutralized by addition of an excess of serum containing medium and the cells centrifuged at 1000rpm for 10 minutes. The cell pellet was resuspended in DMEM/10% FCS, and a single cell suspension prepared by mechanical dissociation and passage through a 70μm cell strainer. Trypan blue excluding cells were counted in a haemocytometer, and cells were plated into poly-D-lysine treated 8-well chamber slides at a density of $2x10^5$ cells/well in Dulbecco's MEM supplemented with 10% FCS. Cultures were incubated for 24 hours at 37°C/5% CO_2, then the medium was replaced with DMEM supplemented with SATO (final concentration; 4.3mg/ml BSA, 0.77μg/ml progesterone, 20μg/ml putrescine, 0.49μg/ml L-thyroxine, 0.048μg/ml selenium and 0.42μg/ml tri-iodo-thyronine).

Cultures were incubated for 5 days, then removed from the incubator and treated with compounds. Jnk inhibitors were added at concentrations ranging from 1nM to 1μM to 4 independent wells per concentration. 15 minutes following addition of Jnk inhibitors, MPP$^+$ was added directly to the wells to give a final concentration in the well of 10μM. 4 wells were treated with MPP$^+$ 10μM alone, and 4 left as untreated controls. zVAD-fmk 300μM and Example 2 500nM were used as positive controls. Once compounds and MPP$^+$ had been added, cultures were returned to the incubator at 37°C/5% CO_2 for a further 48 hours prior to fixation and immunostaining.

2. Determination of TH-immunoreactive cell survival

To determine the numbers of surviving dopaminergic neurons, immunocytochemistry was carried out using a rabbit polyclonal antibody raised against TH. Non-specific binding sites were blocked using 10% normal goat serum in PBS, then primary antibody was added at 4°C overnight. The next day, the cells were washed and treated with biotin conjugated goat anti-rabbit IgG for one hour, followed by peroxidase conjugated avidin biotin complex, both made up from the Vectastain® Elite ABC kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. Staining was visualized using Vector™ SG (Vector Laboratories) insoluble peroxidase substrate according to the manufacturer's instructions. Following staining, the gaskets were removed from the chamber slides, and the slides mounted using aqueous mountant. Slides were blinded by another investigator before quantification of TH-immunoreactive cell survival. To determine TH-immunoreactive cell survival, cells were visualized using transmitted light on a Zeiss Axiovert inverted microscope using a 10X objective. Counts were made of all the TH-immunoreactive cells present in each well.

3. Statistical analyses

Data analysis was performed using a one way analysis of variance, followed by Dunnett's t-test. In each case the data refer to one representative experiment, with four independent replicates for each data point. Significance was reached at $p<0.05$. Data shown are normalized to percentage of control response; all statistical analyses, however, were carried out on the cell counts.

Results

Effects of Example 2

Table 2 shows the effects of Example 2 on survival of mesencephalic dopaminergic neurones exposed to MPP^+. Example 2 causes a maximal effect at 500nM, where survival is restored to 72% of untreated control. Non-specific toxicity is observed with 10μM treatment. Significant increases are observed with concentrations of 10nM and above; at 10μM, however, there is a significant decrease

through non-specific toxicity (* $p<0.05$, ** $p<0.01$). The results shown here are the mean ± standard error margin of three independent experiments.

Table 2

	Example 2	
Treatment	Mean	SEM
Control	100.00	2.98
MPP^+ 10μM	48.77	1.17
1nM	54.72	1.85
10nM	60.77*	2.45
100nM	59.95*	1.51
500nM	72.41**	0.77
1μM	71.14**	2.86
10μM	32.08	3.41

Effects of JNK inhibition on survival of rat superior cervical ganglion neurons.

Summary

Rat superior cervical ganglion (sympathetic) neurons are a population of NGF dependent neurons, which die by apoptosis when deprived of NGF. Activation of c-jun-N-terminal kinase (JNK) has been implicated in apoptosis in sympathetic neurons. An inhibitor of JNK, Example 2, was tested for neuroprotective effects in two models of sympathetic neuronal cell death.

In the first model, ganglia were dissociated and plated directly into culture plates in the presence of compound for 48 hours, then fixed and survival assayed using an ELISA for GAP-43. This model will hereafter be referred to as the 'survival assay'.

In the second model, ganglia were dissociated and plated in the presence of NGF 25ng/ml for 4 days. The NGF was then removed by washing and application of a blocking antibody, and L-790,984 coadministered for 72 hours. Survival was then assayed using the GAP-43 ELISA. This model will be referred to hereafter as the 'NGF deprivation assay'.

At least three experiments were carried out for each model. The results of the ELISA were verified by immunostaining cultures from one sample

experiment and counting surviving sympathetic neurons. In both of these models of sympathetic neuronal apoptosis, inhibition of JNK using Example 2 resulted in a significant increase in sympathetic neuronal survival.

Methods

1. Preparation of superior cervical ganglion neurones

Superior cervical ganglia were dissected from 1 – 3 day old Sprague-Dawley rat neonates. Ganglia were enzymatically dissociated using 0.25% trypsin for 45 minutes. The trypsin was then inhibited using Dulbecco's MEM (DMEM) supplemented with 10% fetal bovine serum, and the cells mechanically triturated using a pipette tip to form a single cell suspension. Neurons in the suspension were counted using a haemocytometer, and plated at a density of 3000 – 5000 neurons per well in poly-D-lysine and laminin coated 96 well tissue culture clusters in DMEM supplemented with B27 serum substitute. Cultures were then incubated at 37°C/5% CO_2. One hour following plating, cultures were either treated with NGF 25ng/ml or with L-790,984 at a range of concentrations for the NGF deprivation and survival assays respectively.

Cultures for the NGF deprivation assay were returned to the incubator for 4 days. Following this, the medium was aspirated, plates washed once with DMEM/B27, and the cultures treated with Example 2 at concentrations ranging from 1nM to 10μM, together with an anti-NGF blocking antibody at 250ng/ml. Cultures were then returned to the incubator for a further 72 hours prior to fixation and survival quantification. Cultures treated with Example 2 immediately for the survival assay were returned to the incubator for 48 hours; cultures were then fixed and survival quantified using the GAP-43 ELISA.

2. GAP-43 ELISA protocol

Cultures were fixed by the addition of an equal volume 4% paraformaldehyde to each well for 10 minutes; this was then aspirated, and replaced by a further volume of 4% paraformaldehyde for a further 20 minutes at room temperature. Plates were then washed three times with PBS/0.3% TX100, and non-specific binding sites blocked by the addition of 5% normal horse serum (NHS) in PBS/0.3% TX100. Plates were incubated at room temperature for one hour, then the blocking serum was aspirated without washing and replaced with primary antibody.

The primary antibody used was a mouse monoclonal antibody raised against Growth Associated Protein 43 (Sigma), prepared at a dilution of 1:500 in PBS/0.3% TX100/5% NHS. Primary antibody was added to all sample wells, with four control wells returned to blocking serum to act as minus primary control. Plates were then refrigerated overnight at 4°C. The next day, plates were washed three times with PBS/0.3 TX100 and secondary antibody added. The secondary antibody used was peroxidase conjugated sheep anti-mouse IgG, and was added at a dilution of 1:1000 in PBS/0.3% TX100/5% NHS. Plates were incubated for 1 hour at room temperature, then washed three times with PBS/0.3% TX100, and K-Blue insoluble peroxidase substrate added for 30 minutes at room temperature. The optical density of the plates was then read at 650nm, and the survival of neurons calculated and expressed as percentage of the control response.

3. Visualisation of sympathetic neurones for cell counting

Cell counts were performed on one sample plate for each model of sympathetic neuronal cell death, by the addition of a tertiary antibody to the plate, followed by avidin-biotin complex and an insoluble peroxidase substrate. Following quantification of optical density, plates were washed three times in PBS/0.3% TX100, and non-specific sites blocked using PBS/0.3% TX100/5% normal rabbit serum ("NRS"). Plates were incubated for one hour at room temperature, then the blocking serum was aspirated and replaced with biotinylated rabbit anti-sheep IgG at a dilution of 1:500 in PBS/0.3% TX100/5% NRS. Plates were incubated in this antibody for 30 minutes, then washed and treated with peroxidase conjugated avidin-biotin complex for a further 30 minutes. Plates were washed and staining visualized using Vector SG insoluble peroxidase substrate. Cell counts were made of immunostained neurons across the whole of the surface of each well of the plate to confirm the ELISA data.

4. Statistical analyses

All statistical analyses were made using one-way analysis of variance, followed by Dunnet's t-test comparing all groups to untreated control in the case of the survival assay with no NGF exposure, and to the response to anti-NGF 250ng/ml in the case of the NGF deprivation assay. In both cases, significance was deemed to have been reached when $p<0.05$. Both the ELISA data and the cell count data refer to the mean ± standard error margin of one typical experiment for both assays.

Results

Example 2 was tested for survival promoting effects in both the sympathetic neuronal survival assay and the NGF withdrawal assay. In both of these models, there was a significant increase in sympathetic neuronal survival as quantified by the GAP-43 ELISA and by cell counts. As shown in Table 3 below, in the survival assay, the response was significant at concentrations of 300nM and above as quantified by ELISA, and at concentrations of 100nM and above as quantified by cell counts. While in the NGF deprivation assay, shown in Table 4 below, the response was significant at concentrations of 500nM and above, as measured by both the ELISA and cell counts.

Table 3

Example 2	ELISA Data			Cell Counts		
Concentration	Mean		SEM	Mean		SEM
Control	149.19		4.75	129.75		17.61
0.01μM	112.10		4.66	115.50		9.02
0.03μM	99.19		10.33	127.25		14.26
0.1μM	131.45		11.37	170.50		13.99
0.3μM	209.68	*	22.98	267.50	**	10.60
1μM	350.81	**	17.94	363.00	**	18.64
3μM	270.16	**	13.07	321.00	**	11.73
NGF 1ng/ml	618.55		43.73	587.00		13.95

Table 3. Effects of Example 2 in the sympathetic neuronal survival assay, measured by both ELISA and cell counts. Data shown are the mean ± S.E.M. of one typical experiment of three performed; the cell count and ELISA data shown are from the same experiment consisting of four independent wells per treatment group. Significant (*$p<0.05$, **$p<0.01$) increases in cell survival compared to untreated control are observed at Example 2 concentrations of 300nM and above in both the ELISA and cell counts. The response declines at concentrations above 3μM (data not shown).

Table 4

Example 2 Concentration	ELISA Data Mean		SEM	Cell Counts Mean		SEM
Anti-NGF 250ng/ml	40.78		3.43	73.50		3.71
0.001μM	35.44		2.39	82.00		3.89
0.01μM	28.20		1.69	81.00		7.83
0.1μM	22.89	**	3.72	53.75		3.75
0.5μM	60.15	**	3.18	140.25	**	11.55
1μM	102.79	**	5.46	200.00	**	12.28

Table 4. Effects of Example 2 in the sympathetic neuronal NGF deprivation assay, measured by both ELISA and cell counts. Data shown are the mean ± S.E.M. of one typical experiment of four performed; the cell count and ELISA data shown are from the same experiment, consisting of four independent wells per treatment group. Significant (**p<0.01) increases in cell survival over cultures treated with the anti-NGF antibody at 250ng/ml alone are observed at Example 2 concentrations of 500nM and 1μM in the cell count data. In the ELISA data, significant increases are observed with Example 2 concentrations of 500nM and 1μM; a significant lowering was observed in the ELISA at 0.1μM, but this effect was not significant when the cell number was quantified by cell counts.

Conclusions

The JNK inhibitor Example 2 was tested in two models of sympathetic neuronal cell death, an NGF deprivation model using a blocking antibody, and a survival model. In both of these models, significant increases in the number of surviving sympathetic neurons were observed, evaluated both by an ELISA to GAP-43, and by cell counts. JNK inhibition, therefore, protects sympathetic neurons against the apoptotic cell death induced by NGF withdrawal in this neuronal population *in vitro*.

Testing of Compounds in Mouse Cerebellar Granule Neurons

Isolation of Cells:

1. Dissect out cerebella from 7-9 day old CD-1 mouse pups; remove meninges.

2. Mince and dissociate with trypsin. Halt trypsinization with Dnase I and egg white trypsin inhibitor.

3. Individual cells are obtained by trituration with a pasteur pipet.

4. Cells were resuspended in cell culture media [(cMEM) E-MEM), 25mM glucose, 10% fetal bovine serum, 2mM glutamine, 100μg/mL gentamycin, 25mM KCl] and seeded at 1.2 X 10^5 cells per well onto 96-well microplates pre-coated with poly-D-lysine.

5. Cultures were incubated at 37°C in 6% CO2, and were used for experiments on day 5-7 *in vitro*.

Detection of neuronal Apoptosis

1. Replace media in column 1 with serum-free cMEM. Replace medium in columns 2-12 with serum-free cMEM with low (5 mM) K+.

2. Add drug titrations (serial diluted in DMSO; final 1% DMSO). Incubate 8h @ 37°C.

3. Spin plate @ 1500rpm 10min., remove media and add lysis buffer.

4. Incubate 30min. room temp. shaking.

5. Spin plate @ 1500rpm 10min., transfer supernatant to fresh plate. Store @ 4°C.

6. Transfer 5μL supernatant and 45μL EIA reagent to EIA strip plate (positive control standards in column 12); incubate @ room temp 2h.

7. Wash strip plate with PBS using the plate washer.

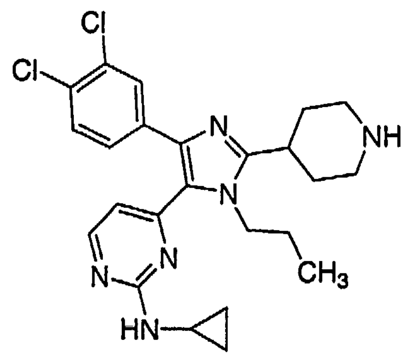
8. Add 150μL K-blue substrate (ELISA Technologies, Inc., Gainsville, FL); stop using 50μL Red Stop. Read plate @ 650nm.

Figure 1 shows the that the IC_{50} of Example 2 for inhibition of neuronal apoptosis from mouse cerebellar granule neurons =100nM.

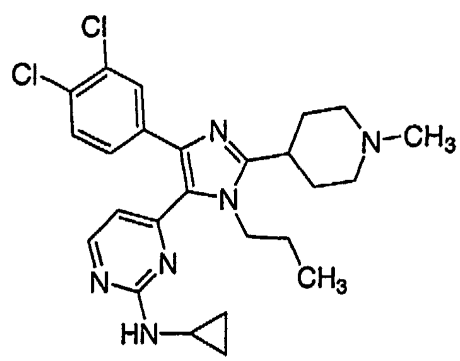
Examples

Compounds utilized in the method of the present invention include:

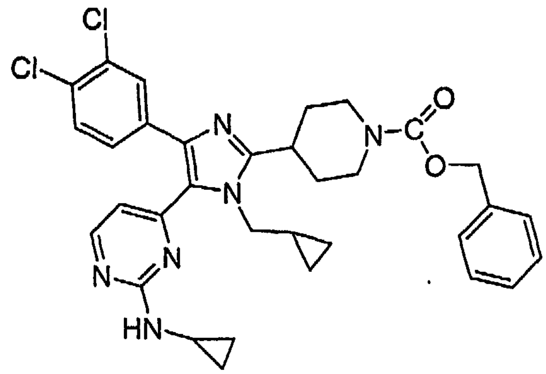
Example 1: Cyclopropyl-{4-[5-(3,4-dichlorophenyl)-2-piperidin-4-yl-3-propyl-3H-imidazol-4-yl]-pyrimidin-2-yl}amine



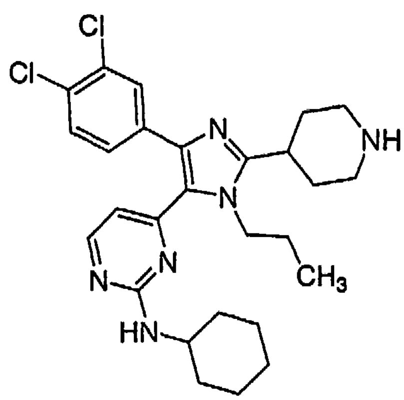
Example 2: Cyclopropyl-{4-[5-(3,4-dichlorophenyl)-2-[(1-methyl)-piperidin]-4-yl-3-propyl-3H-imidazol-4-yl]-pyrimidin-2-yl}amine



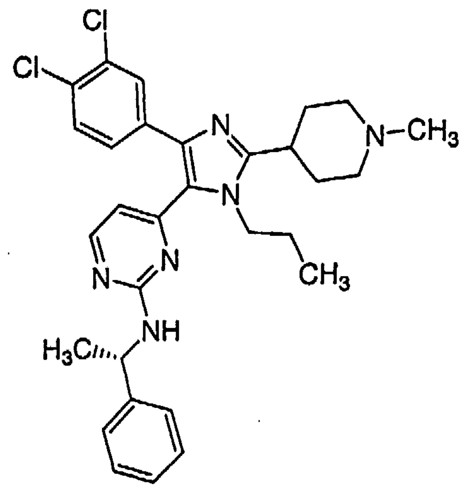
Example 3 :



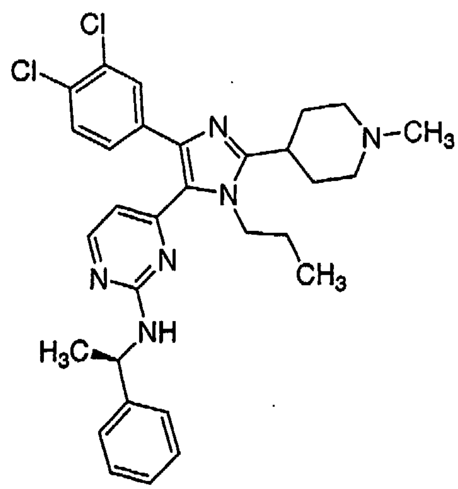
Example 4:



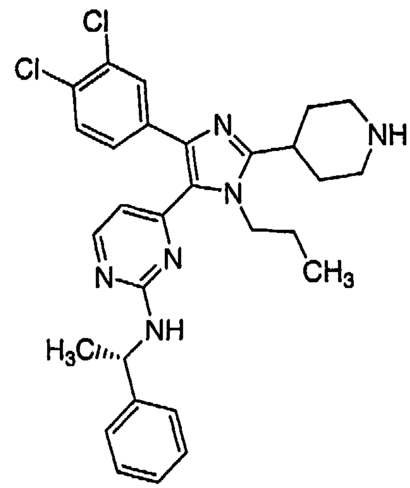
Example 5a:



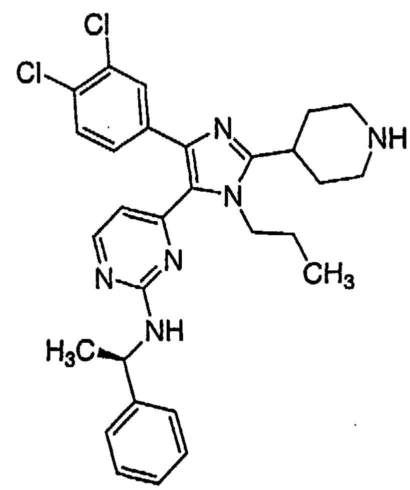
Example 5b:



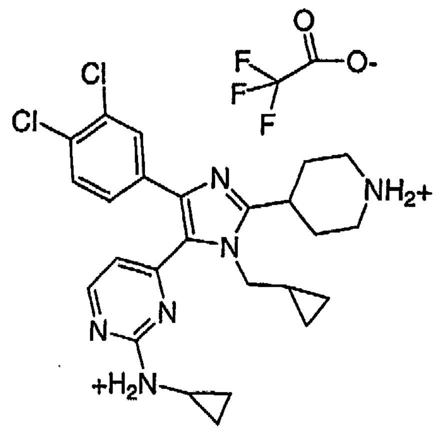
Example 6a:



Example 6b:



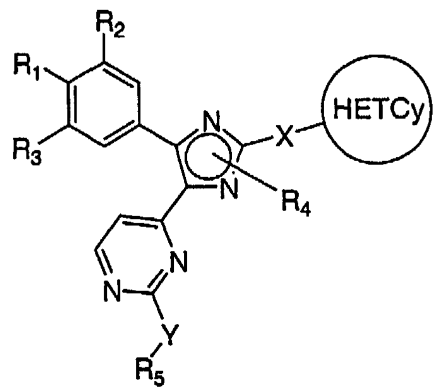
Example 7: Cyclopropyl-{4-[3-cyclopropylmethyl-5-(3,4-dichlorophenyl)-2-piperidin-4-yl-3H-imidazol-4-yl]-pyrimidin-2-yl}amine bis trifluoroacetic acid salt



Other variations or modifications, which will be obvious to those skilled in the art, are within the scope and teachings of this invention. This invention is not to be limited except as set forth in the following claims.

WHAT IS CLAIMED IS:

1. A method of promoting neuronal survival comprising the step of administering an amount of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase:



(I)

wherein

R_1 is –F, –Cl, –Br, -OH, –SH, –NH_2, or –CH_3;

R_2 is –F, –Cl, –Br, -OH, –SH, –NH_2, or –CH_3;

R_3 is –H, -F, –Cl, –Br, -OH, –SH, –NH_2, –CH_3, –OCH_3, or –CH_2CH_3;

R_4 is –C_{1-4}alkyl optionally substituted with a –C_{3-7}cycloalkyl;

R_5 is –C_{1-4}alkyl or –C_{3-7}cycloalkyl, wherein the –C_{1-4}alkyl is optionally substituted with a phenyl;

X is a bond or an alkyl bridge having 1-3 carbons;

Y is –NH– or –NH_2^+–; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with –C_{1-4}alkyl or –C(O)–O–CH_2phenyl.

2. The method according to claim 1, wherein R_1 is –Cl.

3. The method of claim 2, wherein R_3 is –H.

4. The method according to claim 1, wherein X is a bond.

5. The method of claim 4, wherein Y is –NH–.

6. The method of claim 4,
R_5 is $-C_{1-4}$alkyl, optionally substituted with a phenyl; and
Y is –NH–.

7. The method of claim 4, wherein
R_5 is $-C_3$cycloalkyl; and
Y is –NH–.

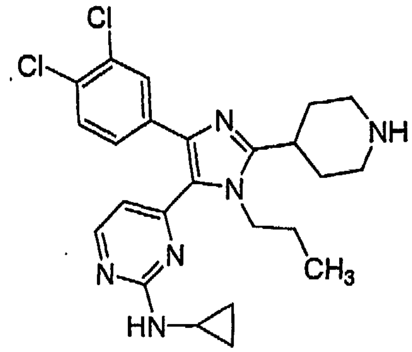
8. The method of claim 4, wherein
R_5 is $-C_6$cycloalkyl; and
Y is –NH–.

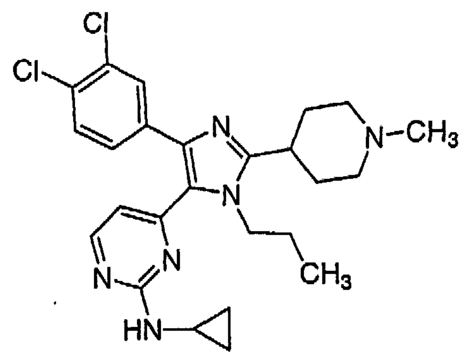
9. The method of claim 4, wherein
R_5 is $-C_3$cycloalkyl; and
Y is $-NH_2^+-$.

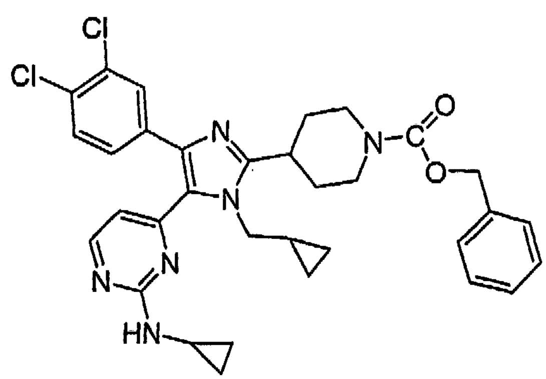
10. The method according to claim 1, wherein said pharmaceutically acceptable salt is a bis trifluoroacetic acid salt of a compound represented by Formula (I).

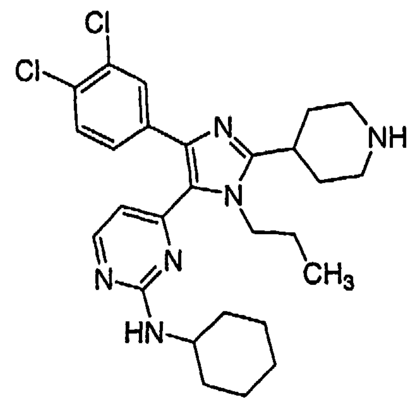
11. The method according to claim 1, wherein HETCy is a 5-6 membered non-aromatic heterocycle with 1-2 nitrogen atoms contained therein.

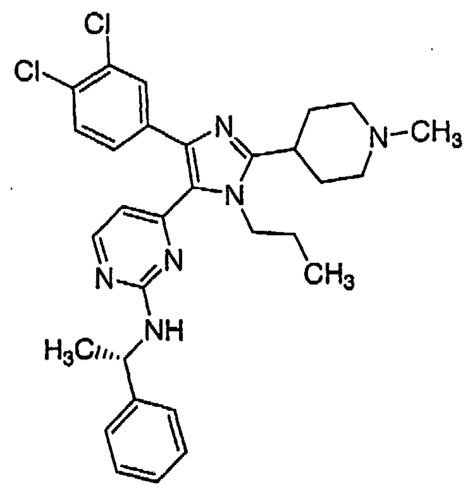
12. The method according to claim 1, wherein said compound represented by Formula (I) is

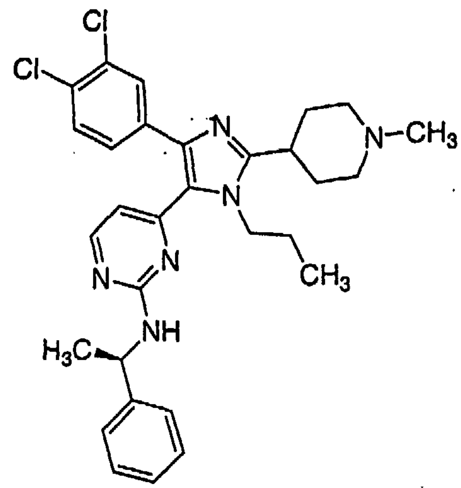


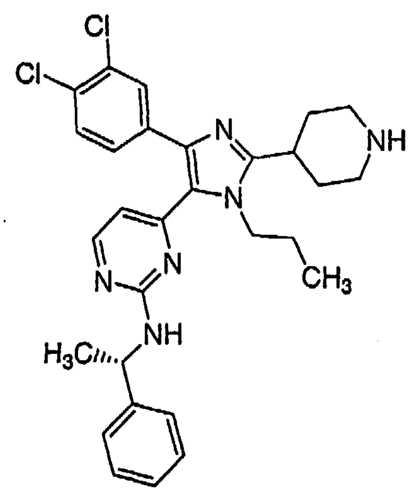


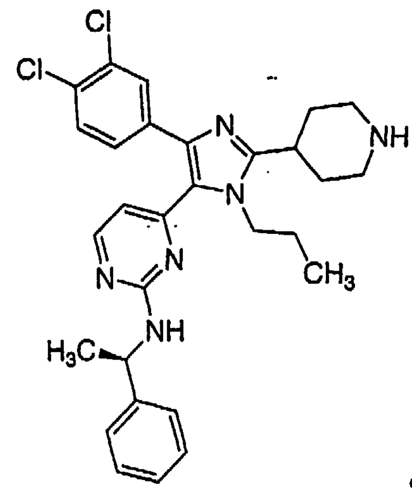




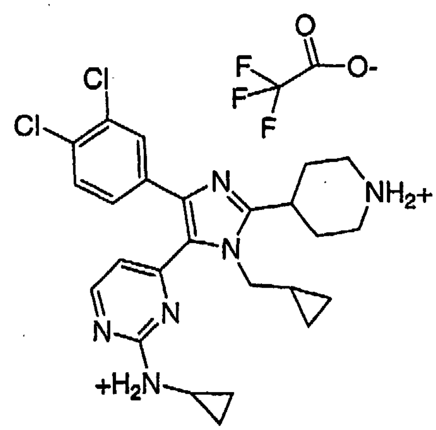






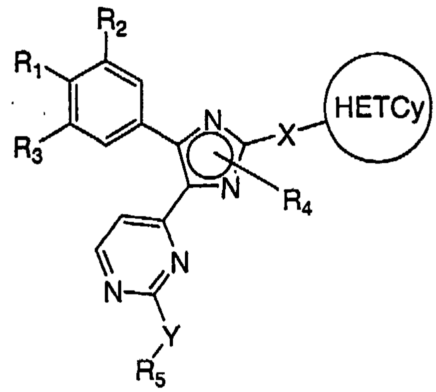


or



13. A method of promoting neuronal survival comprising the step of administering a therapeutic amount of a composition, said composition comprising:

a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase:



(I)

wherein

R_1 is –F, –Cl, –Br, -OH, –SH, –NH_2, or –CH_3;

R_2 is –F, –Cl, –Br, -OH, –SH, –NH_2, or –CH_3;

R_3 is –H, –F, –Cl, –Br, -OH, –SH, –NH_2, –CH_3, –OCH_3, or –CH_2CH_3;

R_4 is –C_{1-4}alkyl optionally substituted with a –C_{3-7}cycloalkyl;

R_5 is –C_{1-4}alkyl or –C_{3-7}cycloalkyl, wherein the –C_{1-4}alkyl is optionally substituted with a phenyl;

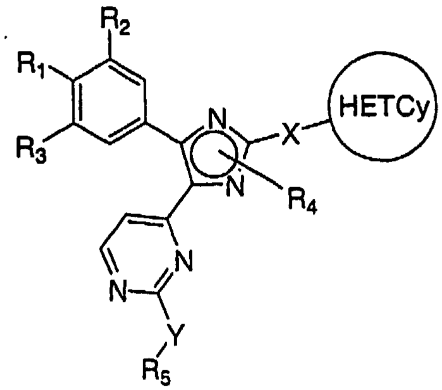
X is a bond or an alkyl bridge having 1-3 carbons;

Y is –NH– or –NH_2^+–; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with –C_{1-4}alkyl or –C(O)–O–CH_2phenyl; and

a pharmaceutically acceptable carrier.

14. A method of treatment or prevention of stroke, Parkinsons disease, Alzheimer's disease, amyotrophiclateral sclerosis, multiple sclerosis, spinal cord injury, head trauma, and seizure comprising the step of administering a therapeutically effective amount, or a prophylactically effective amount, of a compound represented by Formula (I), or a pharmaceutically acceptable salt thereof, effective to inhibit the activity of c-jun-*N*-terminal kinase:



(I)

wherein

R_1 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_2 is –F, –Cl, –Br, -OH, –SH, $-NH_2$, or $-CH_3$;

R_3 is –H, –F, –Cl, –Br, -OH, –SH, $-NH_2$, $-CH_3$, $-OCH_3$, or $-CH_2CH_3$;

R_4 is $-C_{1-4}$alkyl optionally substituted with a $-C_{3-7}$cycloalkyl;

R_5 is $-C_{1-4}$alkyl or $-C_{3-7}$cycloalkyl, wherein the $-C_{1-4}$alkyl is optionally substituted with a phenyl;

X is a bond or an alkyl bridge having 1-3 carbons;

Y is –NH– or $-NH_2^+-$; and

HETCy is a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, optionally containing 1-2 additional N atoms and 0-1 O or S atom, and optionally substituted with $-C_{1-4}$alkyl or $-C(O)-O-CH_2$phenyl.

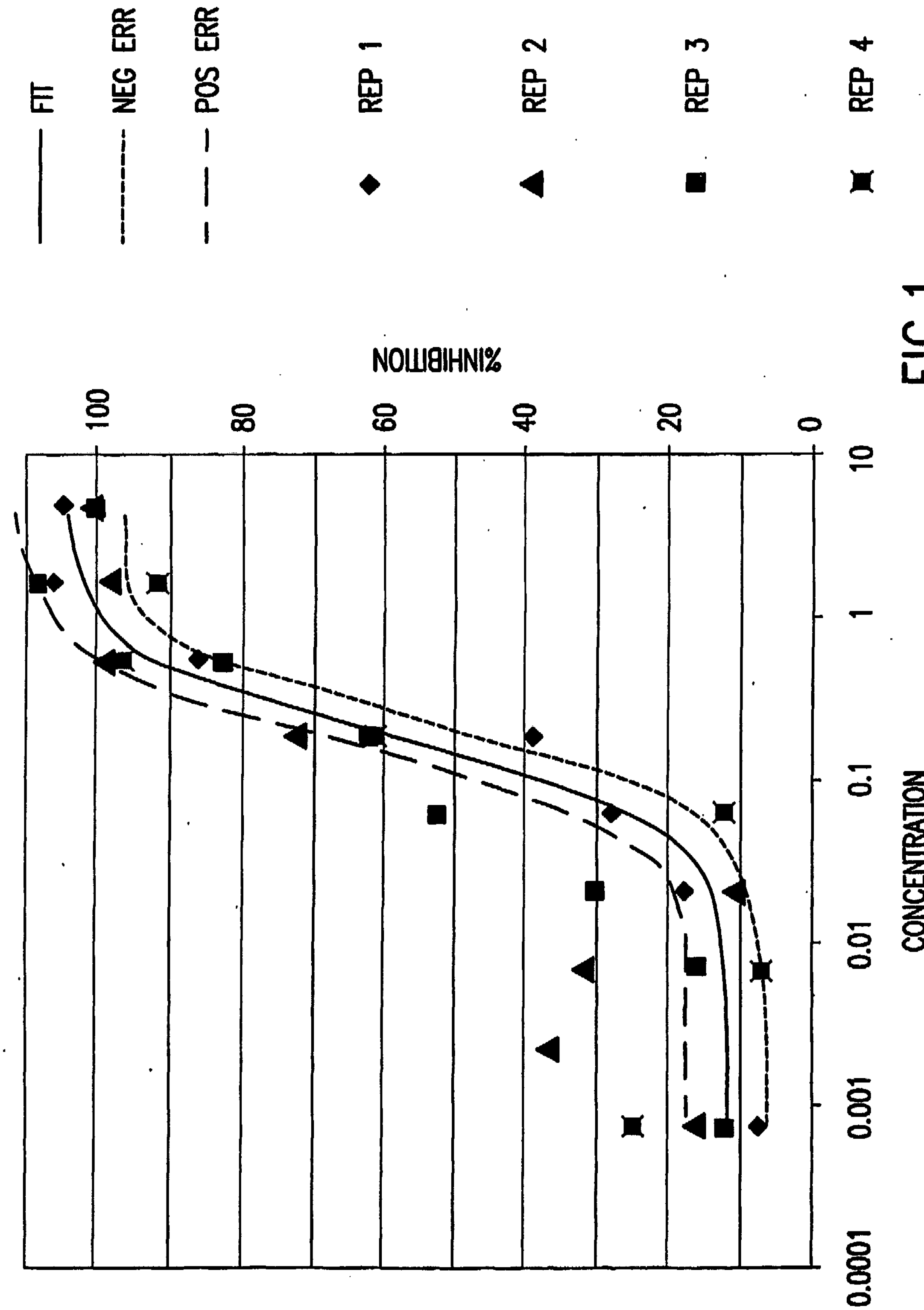

FIT
NEG ERR
POS ERR
REP 1
REP 2
REP 3
REP 4
%INHIBITION
100
80
60
40
20
0
10
1
0.1
0.01
0.001
0.0001
CONCENTRATION

FIG.1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/17018

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/415
US CL :514/396, 397
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/396, 397

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CHEMICAL ABSTRACTS, MED LINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,736,381 A (DAVIS et al) 07 April 1998, see entire document.	1-14
A	US 5,783,664 A (LEE et al) 21 July 1998, see entire document.	1-14
A	US 5,804,427 A (DAVIS et al) 08 September 1998, see entire document.	1-14
A	US 5,859,041 A (LIVERTON et al) 12 January 1999, see entire document.	1-14
A	US 5,955,366 A (LEE et al) 21 September 1999, see entire document.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 29 AUGUST 2001	Date of mailing of the international search report 26 SEP 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JAMES H. REAMER Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*